Standard Guide for Microspectrophotometry and Color Measurement in Forensic Paint Analysis[1]

This standard is issued under the fixed designation E2808; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

Color is one of the most important comparative characteristics of paints. The comparison of color is one of the first steps taken in a forensic paint comparison and it is essential to note that this guide does not propose the use of instrumental color comparison for objects that are distinguishable to the eye. Since the 1940s, analytical instruments have been able to discriminate colors that the average human eye cannot distinguish. Microspectrophotometers (MSPs), in particular, allow for an objective measurement of the color of small, millimetre or submillimetre samples and are more precise or quantitative compared to the more subjective results of visual microscopical color comparisons.

Suitable instruments with appropriate optics, sensitivity, resolution, and dynamic range can measure and produce spectral curves of light energy from small samples as that light is transmitted, absorbed, or reflected by the sample. These spectra are collected over small measurement steps or increments of one-half to a few nanometers each. MSPs typically operate in the visible spectral region (~380 to ~800 nm) and also in the ultraviolet region (~190 to ~380 nm). They should not be confused with broadband or absorption filter-based, tristimulus systems or low-resolution, large measurement step (5 nm or more) spectral analyzers.

The spectral limits of different instruments can vary in all of the above noted spectral regions and may also include the near infrared region from about 780 to 2100 nm. The usefulness of this last spectral region in the analysis and comparison of paint fragments is currently indeterminate and will not be covered in this guide.

Subjective terms such as "blue, "violet," or "purple" are inadequate descriptors of actual color. MSP instruments can be used to describe or numerically "name" an item's color by calculating the item's colorimetric values. These values, or chromaticity coordinates, can be expressed in any one of several coordinate systems and are useful in the development and maintenance of color comparison databases.

Colorimetric values are of limited use in actual color comparison of evidence samples because differing spectral curves can yield identical colorimetric values. This is commonly found in industrial or commercial paint-matching protocols in which the only requirement is to paint an item so it appears to be the same as others. The eye's perception and the colorimetric values of two items may indicate that they are the same color, but the spectral curves of those items may still be distinguished. This leads to the use of the MSP in the comparison of visually indistinguishable colored items.

1. Scope

1.1 This guide is intended to assist individuals and laboratories that conduct forensic visible and ultraviolet (UV) spectral analyses on small fragments of paint using Guide E1610.

1.2 This guide deals primarily with color measurements within the visible spectral range but will also include some details concerning measurements in the UV range.

1.3 This guide does not address other areas of color evaluation such as paint surface texture or paint pigment particle size, shape, or dispersion within a paint film that are evaluated by other forms of microscopy. Other techniques such as spectral luminescence, fluorescence, and near infrared (NIR) are not included in this guide because of their limited use, lack of validation, or established efficacy in forensic paint analysis.

1.4 This guide is directed at the color analysis of commercially prepared paints and coatings. It does not address the analysis or determination of provenance of artistic, historical, or restorative paints, but it may be found useful in those fields.

---

[1] This guide is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.

Current edition approved March 1, 2011. Published May 2011. DOI: 10.1520/E2808-11.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D16 Terminology for Paint, Related Coatings, Materials, and Applications
D1535 Practice for Specifying Color by the Munsell System
D2244 Practice for Calculation of Color Tolerances and Color Differences from Instrumentally Measured Color Coordinates
E179 Guide for Selection of Geometric Conditions for Measurement of Reflection and Transmission Properties of Materials
E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers
E284 Terminology of Appearance
E308 Practice for Computing the Colors of Objects by Using the CIE System
E805 Practice for Identification of Instrumental Methods of Color or Color-Difference Measurement of Materials
E1610 Guide for Forensic Paint Analysis and Comparison

3. Terminology

3.1 *Definitions*—For definitions of paint-associated terminology used in this guide, see Terminologies D16, E284, and E1610.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *absorbance, n*—logarithm to the base 10 of the reciprocal of spectral transmittance, (T).

$$A = \log_{10}(1/T) = -\log_{10}T$$

3.2.1.1 *Discussion*—It is often expressed as a fraction or decimal value and may be referred to as optical density.

3.2.2 *absorption, n*—transformation of incident energy into longer wavelength energy.

3.2.3 *bandwidth, n*—optical width of the monochromator exit slit or the optical width of a semiconductor detector element that will vary with monochromator design.

3.2.3.1 *Discussion*—This term can also refer to the wavelength interval over which radiant energy is greater than 50 % of the maximum intensity.

3.2.4 *charge-coupled device (CCD), n*—a silicon-based semiconductor chip consisting of a two-dimensional matrix of photo sensors or pixels.

3.2.5 *chromaticity, n*—dimensions of a color stimulus, excluding luminous intensity, and expressed in terms of hue and saturation (Commission Internationale de l'Eclairage (CIE)) or redness-greenness and yellowness-blueness (L*, a*, b*).

3.2.5.1 *Discussion*—It is generally represented as a point in a constant luminance plane of a three-dimensional color space.

3.2.6 *chromaticity coordinates, CIE, n*—ratios of each of the three tristimulus values X, Y, and Z in relation to the sum of the three designated as x, y, and z, respectively.

3.2.6.1 *Discussion*—They are sometimes referred to as the trichromatic coefficients. When written without subscripts, they are assumed to have been calculated for Illuminant C and the 2° (1931) Standard Observer unless specified otherwise. If they have been obtained for other illuminants or observers, a subscript describing the observer or illuminant should be used. For example, x_{10D} and y_{10D} are chromaticity coordinates for the 10° observer and Illuminant D. A "standard observer" refers to specific numerical values that represent the nominal color response of the human eye to different wavelengths of light. It is based on a study of the average retinal response of the human population.

3.2.7 *chromaticity diagram, CIE, n*—two-dimensional graph that describes a color as the locus of chromaticity coordinates in a field of monochromatic light varying from 380 to 780 nm in wavelength where X is the abscissa of the coordinate system and Y is the ordinate and it is used to describe and compare the colors of both luminous and non-luminous materials.

3.2.8 *CIELAB Unit E, n*—(L*a*b*) color difference equation from Practice D2244.

3.2.8.1 *Discussion*—The color difference E between two samples is given by:

$$\Delta E_{CIE}(L^*, a^*, b^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where

L* (pronounced "L star") = where a color falls on a vertical scale from light to dark,
a* = where the color falls on the continuum of colors between red and green, and
b* = where the color falls on the continuum of colors between blue and yellow.

3.2.9 *colorimetry, n*—conversion of instrumental light measurements into psychophysical descriptions or numerical notations that can be correlated with visual evaluations of color and color differences.

3.2.10 *effect pigment, n*—any paint pigment that is designed to produce a significant change in color attribute(s) in a paint film when the film is viewed or illuminated from varied geometries.

3.2.11 *error ellipse (visual limits), n*—plotted limits of variation in visually indistinguishable color coordinate values that form an elliptical shape around a central color value when plotted in a chromaticity diagram.

3.2.12 *grating, n*—parallel set of linear, regularly repeating structures that, when illuminated, produces maxima and minima of light intensity as a consequence of interference.

3.2.12.1 *Discussion*—These maxima and minima vary in

---

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

position with wavelength. This allows radiation of any given wavelength to be isolated from a complex mixture of wavelengths and allows the grating to be used as a monochromator.

3.2.13 *illumination aperture, n*—element in the optical path of a microspectrophotometric (MSP) system that limits the area of illumination reaching the sample focal plane.

3.2.14 *measuring aperture, n*—element in the optical path of a microspectrophotometric (MSP) system that limits the area of illumination reaching the detector focal plane.

3.2.15 *monochromator, n*—device designed to isolate narrow wavelength ranges of light from complex, broad-spectrum radiation.

3.2.16 *photomultiplier tube (PMT), n*—photosensitive vacuum tube device that quantitatively converts photons of light into electrical energy.

3.2.17 *reflectance, R, n*—the ratio of the radiant power reflected by the specimen to the radiant power incident on the specimen.

3.2.17.1 *Discussion*—In popular usage, it is considered as the ratio of the intensity of reflected radiant energy from a test sample to that reflected from a defined reference standard.

3.2.18 *significant difference, n*—difference between two specimens that indicates their possible common origin cannot be established.

3.2.19 *standard observer, CIE, n*—visual color perception data set adopted by CIE to represent the response of the average human eye when light adapted to an equal energy spectrum.

3.2.19.1 *Discussion*—Unless otherwise specified, the term applies to the data adopted in 1931 for a 2° field of vision. The data adopted in 1964, sometimes called the 1964 observer, were obtained for a 10° field of vision and are generally used in industrial measurements.

3.2.20 *standard illuminant, CIE, n*—standard sources for which the CIE specified the spectral energy distribution as follows:

3.2.20.1 *standard illuminant A, CIE, n*—tungsten filament lamp operated at a color temperature of 2856 K, approximating a blackbody operating at that temperature.

3.2.20.1.1 *Discussion*—It is defined in the wavelength range of 380 to 770 nm.

3.2.20.2 *standard illuminant C, CIE, n*—approximation of overcast daylight having a correlated color temperature of approximately 6770 K.

3.2.20.2.1 *Discussion*—This color of light can be obtained by using a combination of Illuminant A and a color-correcting filter. It is defined in the wavelength range of 380 to 770 nm.

3.2.20.3 *standard illuminant D, CIE, n*—approximation of bright daylight illumination having a correlated color temperature of 6504 K in the spectral range of 300 to 830 nm.

3.2.20.3.1 *Discussion*—The UV portion of Illuminant D, 300 to 380 nm, is necessary to describe correctly colors that contain fluorescent colorants or ultraviolet (UV) absorbers. The UV and visible portions are designated as UVD and VisD. Illuminant D is based on actual measurements of the spectral distribution of daylight and is the most commonly used standard illuminant for spectral measurements.

3.2.21 *thermochromy, n*—characteristic of some materials, including some pigments, to change color as temperature changes.

3.2.22 *step width, n*—distance between two points of wavelength measurement in a spectrum, not to be confused with resolution although it can have an impact on resolution.

3.2.23 *transmittance, T, n*—the ratio of radiant power transmitted by the specimen to the radiant power incident on the specimen.

3.2.23.1 *Discussion*—Both absorption and reflection influence total transmittance.

3.2.24 *tristimulus values, CIE, n*—amounts (in percent) of the three components necessary in a three-color additive mixture required for matching a color.

3.2.24.1 *Discussion*—These components are designated as X, Y, and Z in the CIE system. The illuminant and standard observer color-matching functions must be designated. If they are not, an assumption is made that the reported values are for the 1931 observer (2° field) and Illuminant C. The values obtained depend on the method of integration used, the nature of the sample surface, sample homogeneity, and the instrument design. Tristimulus values are therefore not absolute values for a sample, but relative values dependent on the method used to obtain them. Examples of calculations of tristimulus values in the CIE system can be found in Practice E308.

3.2.25 *Wood's anomaly, n*—effect caused by a polarization of diffracted energy as a result of non-uniform reflection as the angle of incidence varies on a holographically blazed monochromator.

3.2.25.1 *Discussion*—This effect can be observed as a distinct transmittance maximum within the spectrum.

4. Summary of Practice

4.1 One of the most obvious decorative properties of paint is its color. Usually, one or more major pigments are used in a paint together with one or more minor pigments whose purpose is to modify color or other film properties. The interaction of pigments with light is very complex with light being scattered, absorbed, and reflected within the paint layer.

4.2 The processes responsible for producing the color of pigments include: crystal-field effects with transition metal compounds (most of the inorganic pigments belong to this group), electron transitions between molecular orbitals in compounds with conjugated double bonds (organic pigments), electron transitions in compounds with conduction energy bands (metal pigments and pure semiconductors), and other processes caused by geometrical and wave optics (interference, scattering, diffraction in optically variable pigments, liquid crystal pigments, and so forth). All of these processes are based on the interaction between ultraviolet-visible (UV-Vis) light waves themselves or with electrons in pigments and can be measured and compared using MSP.

4.3 The analysis of paint using MSP can be carried out using either transmission or reflectance techniques. However, be aware that paint color comparisons or pigment identifications using reflectance measurements are difficult tasks because of the effects of surface observation angle, surface texture, and sample curvature. This method is seldom used for detailed color comparison or pigment identification, but it can be useful in exclusionary comparisons of bulk colors.

4.4 *Basic Principles:*

4.4.1 A MSP can measure reflected or transmitted radiation at selected wavelengths. The resulting total spectral curve of a colored sample is produced by comparing the light reflected from a sample to the light reflected from a white reflectance standard or by comparing light transmitted by the sample to the light transmitted by a colorless background.

4.4.2 Paint colors are usually measured in transmission through thin sections. Reflectance techniques can also be used especially when fluorescence measurements in the UV range are desired. When performing measurements on UV-absorbing materials in the UV region, transmission techniques are recommended.

4.4.3 In reflectance measurements of paint color, a sample is illuminated and the fraction of light reflected in the visible range is recorded. The reflectance curve obtained provides a representation of the color. In transmission measurements, a paint thin section is illuminated and the fraction of light transmitted or absorbed in the Vis or UV range or both is recorded.

4.4.4 Transmittance spectra may be plotted in either percent transmittance or absorbance. Reflectance spectra may be plotted in either percent reflectance or absorbance.

4.5 *Colorimetry:*

4.5.1 To achieve accurate color values, a number of instrumental parameters have to be defined. In 1931, CIE defined the standard illuminants, measuring conditions and the three standard primary colors [*X*], [*Y*], and [*Z*]. The amount of each of these colors needed to match a specific color can be calculated. These calculations yield a mathematical representation of a color (tristimulus values). This is described in Practice D1535.

4.5.2 Using a mathematical equation, these tristimulus values can be converted into L*, a*, and b* coordinates. The Color Measurement Committee of the Society of Dyers and Colorists recommends this system for the description of color. From the values of L*, a*, and b*, color differences can be obtained in CIELAB units. See Practice E805.

4.6 *Metamerism*—Two samples can appear to have the same color under one type of illumination but may appear dissimilar under different lighting conditions. This phenomenon is one form of metamerism. A second form occurs when two samples appear to be the same color under all lighting conditions, yet their reflectance/transmittance curves are different. Both forms are a result of differences in their pigment compositions. Note that two objects with different reflectance or transmittance curves can have identical colorimetric values (tristimulus values or chromaticity coordinates). Therefore, it is not recommended to use colorimetric values alone for detailed sample comparisons.

4.7 *Instrumentation:*

4.7.1 The MSP has been designed to meet the requirements of analyzing the color of a sample in situ. In scanning spectroscopy, a MSP consists basically of a research-grade microscope with a stabilized light source, a spectrometer, and a data-processing device. The main function of the microscope is to reflect or transmit light energy efficiently, uniformly, and reproducibly. The spectrometer contains a spectrally dispersive element, most commonly a diffraction grating, and a detector and measures intensity of light energy with respect to spectral position within a spectral range. All MSPs are single-beam instruments: a standard or a blank is measured, the result is stored, and a sample is measured and a ratio made to the blank to yield a transmittance or reflectance spectrum. MSP systems are generally used in forensic analysis because of the small sample sizes presented by paint film fragments, but instruments for color measurement from large samples (spectrometers) can also be used for compiling color databases. Large area measurements can differ from microscale measurements because of the effects of averaging. It can be difficult to compare data from a MSP to that of a macroscale spectrometer.

4.7.2 A monochromator produces monochromatic light of a certain spectral bandwidth. The sample is scanned step by step and a spectrum created point by point. This type of instrument is known as a scanning spectrometer and generally uses a photomultiplier tube (PMT) detector. In a multichannel spectrometer, however, all spectral regions are detected simultaneously and a semiconductor detector is commonly used.

4.7.3 The use of semiconductor detectors is relatively new in forensic analysis. Spectrometers using these devices are composed of a diffraction grating together with an array detector that acts as the photosensitive device. The detector's resolution will depend on the number of diodes or elements in the array, the dispersion and line spacing of the grating, and the distance between the grating and the array. Measurement time is drastically reduced compared to PMT systems because of simultaneous detection of the full spectral range.

4.7.4 Commercially available MSPs have incorporated the requirements of operational settings, data control, processing, and recording with the support of a computer processor. As such, they all carry their own software routines to cope with the tasks.

5. Significance and Use

5.1 Paint sample colors can be measured by reflectance (visible range) or transmission (UV-Vis) for comparison purposes. Transmission measurements are especially necessary for the analysis of UV absorbers in clear coats, the identification of pigments, and the detailed analysis of effect pigments that are not opaque.

5.2 This guide is designed to assist an analyst in the selection of appropriate sample preparation methods and instrumental parameters for the analysis, comparison, or identification of paint pigments and colors.

5.3 It is not the intention of this guide to present comprehensive theories and methods of MSP. It is necessary that the analyst have an understanding of UV-VIS MSP and general concepts of specimen preparation before using this guide. This information is available from manufacturers' reference materials, training courses, and references such as "Visible Microscopical Spectrophotometry in the Forensic Sciences"[3] and "The Role of Colour and Microscopic Techniques for the Characterisation of Paint Fragments."[4]

6. Procedure

6.1 *Reflectance Test Specimens:*

6.1.1 Carry out surface reflectance measurements on clean and undamaged sample areas. Clean the surface of the sample carefully with alcohol to make it dust free. Measurements are usually conducted without further preparation of the sample surface so that surface features that might distinguish between samples are not altered. In some cases, such as in the determination of a vehicle's origin in a hit-and-run case, it is necessary to remove surface features. Surface weathering can be removed by polishing the paint using a diamond paste or another polishing medium so the measured properties of the surface are comparable to data for new, undamaged paint surfaces.

6.1.1.1 Edge-mounted sample reflectance measurements require that the paint edge be carefully polished to a surface finish approaching the size of the smallest pigment particles in the paint layer(s). Prepare samples for comparison in the same manner. Simplify surface finish reproduction by mounting and polishing edge-mounted known and questioned samples side by side.

6.1.2 To obtain optimal results during color measurements, the surface shall be free of scratches, dirt, blemishes and mounted normal to the optical axis of the microscope. The paint sample surface shall be in sharp focus. The area measured shall be as large as possible to average any heterogeneity present. This is particularly important for paints containing effect pigments.

6.1.3 Embed paint samples that are very small or difficult to handle in a resin so that a cross section of the paint can be examined. Before measurement, expose the embedded sample and, when necessary, polish. The MSP measuring field shall fall within the boundaries of the paint layer being analyzed to avoid interference from adjacent layers.

6.1.4 Reflectance measurements are not recommended with very small samples of effect paints because their limited area does not allow for multiple measurements that would provide a representative spectral response curve. In this case, thin cross sections shall be prepared for analysis by transmission methods.

6.1.5 Smeared paints are unsuitable for reflectance measurements, but may be analyzed by transmission methods. (See 6.2.1.1.)

6.2 *Transmission Test Specimens:*

6.2.1 For transmission measurements, thin sections shall be prepared and examined using Vis or UV-Vis spectroscopy. The MSP measuring field shall fall within the boundaries of the paint layer being analyzed to avoid interference from adjacent layers. The area measured shall be as large as possible to average any heterogeneity present.

6.2.1.1 Transmission measurements may be conducted on paint smears. This analysis may include an overall area within the smear or individual pigment particles. If the smeared paint is a mixture, different portions of the smear can be separated and mounted for analysis. These results can provide conclusive exclusionary data but may not provide meaningful associative information.

6.2.2 Measurements in the UV range require quartz or mirror optics, quartz slides and cover slips, and a nonfluorescing, UV-transparent mounting medium such as glycerol.

6.2.3 Paint sections shall be approximately 3-µm thick for UV-Vis measurements of pigmented layers. Because of the low concentration of UV absorbers in clear coats, approximately 20-µm thick sections might be required for analysis with some instruments. To prevent discrepancies in measurements caused by differences in section thickness, embed the questioned and known samples side by side and sectioned together.

6.2.3.1 Photometric (quantitative) reproducibility may be difficult to achieve when thin peels of paint layers and hand-cut cross sections are used for analysis. This may not be problematic when spectral curves exhibit exclusionary features, but it can lead to difficulties in interpretation of shade differences.

6.2.3.2 During UV analyses, a system with a monochromator located in the illumination beam path ahead of the specimen will protect the specimen against overheating and UV degradation. Semiconductor detector systems typically do not experience this problem as they have short measurement times.

6.2.4 A high-magnification, oil immersion objective (about 100×) and condenser are usually necessary for the measurement of small individual pigment particles or particles within pigment aggregates. Dry 100× objectives are available, but their resolution and light-gathering power may be too low for some measurements.

6.2.5 Pigment identification by color analysis usually requires that a spectral library be prepared on the instrument in use. Second-party libraries are suitable for preliminary identifications or classifications. It is recommended that a known sample of the suspected pigment be analyzed before making an identification.

6.3 *General Recommendations:*

6.3.1 To demonstrate the range of variation present in a paint sample, it is suggested that at least five spectra be collected from each non-effect paint film and up to ten spectra for effect finishes, each representing different areas of the sample. This number can be adjusted according to circumstances and is dependent on the degree of spectral consistency observed. Small sample size or poor sample conditions can preclude the acquisition of sufficient spectra to meet these criteria.

6.3.2 A general discussion of geometric considerations for reflectance and transmission measurements is found in Guide E179.

---

[3] Saferstein, *Handbook of Forensic Science*, Vol 1, 2nd edition, Chapter 6, Prentice Hall, 2000.

[4] Caddy, *Forensic Examination of Glass and Paint, Analysis and Interpretation*, Chapter 8, Taylor and Francis, 2001.

7. Instrument Calibration

7.1 Calibration ensures that an instrument is operating within required standards and any errors that might affect data or analytical conclusions are known, limited, accounted for, or corrected. It is necessary to demonstrate that the instrument is calibrated and maintain a record of the calibration results.

7.2 To ensure that historical calibration data are comparable, the optical system shall be optimized each time the system is calibrated. If possible, it is recommended that Köhler illumination be used.

7.3 Define normal operating parameters. The lamp shall be allowed to warm up and stabilize according to manufacturers' instructions or laboratory experience, whichever is longer. Calibration shall not take place until this condition is met. The lamp, the lamp power supply, dispersive system, and detector all need to be thermally stabilized.

7.4 It is essential to demonstrate wavelength and absorbance/photometric accuracy by calibration, such as that described in Practice E275. If colorimetry is used as a comparative feature, then that too shall be calibrated using National Institute of Standards and Technology (NIST) or other traceable standards.

7.5 Wavelength accuracy over the UV-Vis range shall be routinely checked with the aid of NIST-traceable holmium, erbium, or didymium oxide filters, using the working instrument resolution. It is also recommended that wavelength accuracy be periodically checked at the instrument's highest resolution. These measurements shall be carried out in transmission. As exact positions of the absorption bands vary from one batch to another, it is important that the filters be traceable. Resolution can also be checked routinely using these filters.

7.6 Use traceable neutral density filters to demonstrate the photometric accuracy of the system. It is especially important for colorimetric measurement to demonstrate that the instrument's photometric response is linear. Köhler illumination shall be set up before calibration checks are done. A typical set of neutral density calibration filters might include filters of 0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 absorbance units. Quartz filters shall be used for measurements in the UV range.

7.7 Instrument long- and short-term stability/consistency may be demonstrated using the 100 % transmittance line by measuring the ratio of two spectra of a clear and colorless slide/mountant/cover slip preparation. The 100 % line can also be evaluated in reflectance by measuring a white standard such as $BaSO_4$, MgO or commercial grade opal glass.

7.8 Suitable colored standards may be used for checking the overall operation of the equipment and the software's colorimetry calculation program.

7.9 All measurements shall be carried out under similar conditions. These conditions shall include the length of time that a sample resides in the illumination beam. Sample temperature can increase with examination time as it absorbs heat, and the thermochromy of some pigments may result in distinct color differences with temperature changes of only a few degrees.

7.10 The generally used illumination/measuring geometry in reflectance analysis of 45°/0° can be best achieved with the help of a darkfield/brightfield illuminator fitted with darkfield objectives. Some older systems may include dual reflection illumination/measuring objectives to satisfy this geometry.

7.11 Background/system/reference transmittance spectra shall include the absorbance contribution of all system components except the sample of interest (Unimpeded (open air) background measurements are usually difficult to reproduce with microscopical systems that are designed to focus through sample preparations). These components include the microscope slide, cover slip, and mounting medium. The resulting spectra can be used to monitor illuminator performance and warn of unsuitable system adjustments remaining from prior analyses.

7.12 Measuring the ratio of two successive background spectra yields a 100 % line. The transmittance at all wavelength steps will usually be about 100 ± 2 %. If a greater deviation from the 100 % line is observed, it may indicate an instability of the illumination system (especially a xenon lamp) or could indicate the need for slower scanning speed or longer spectral integration times. The 100 % line also offers documentation of a system's basic noise level and operational limits.

7.13 It is recommended that calibration procedures be undertaken and recorded for historical reference approximately once a month or just before analyses, whichever time interval is longer. It is also recommended that a calibration be performed after any maintenance. A historical record of this data cannot only offset court challenges but also monitors system performance and provides an operator with an early warning of system trends and possible deterioration.

8. Instrument Setup and Scanning Parameters

8.1 Measurement ranges may be adjusted as necessary. The wavelength range selection should reflect the maximum amount of information needed for a particular examination. Depending on the application and the instrument used, it will be in the region of ~200 to 780 nm (transmission UV-Vis), ~380 to 780 nm (Vis reflectance and colorimetry), and ~240 to 400 nm (transmission UV). The actual range(s) selected will vary with instrument design, noise level limits, and the dynamic range of the detector.

8.2 The resolution of some instruments can be adjusted. Resolution is usually set higher for transmission measurements (that is, 2.5 nm or less) than for reflectance measurements (that is, approximately 10 nm). This is due to the generally narrower spectral features present in transmittance spectra. High-resolution scans can be used for both types of measurements if desired, but signal-to-noise ratios may deteriorate to unacceptable levels at unnecessarily high-resolution settings.

8.3 Illumination field, measuring aperture, and magnification must be adapted to the sample under investigation.

8.3.1 Set the measuring aperture as large as possible for a given sample and center it on the optical axis of the spectrometer system to improve signal-to-noise ratio and reduce the possible effects of sample heterogeneity.

8.3.2 When possible, set the illumination aperture (field aperture) to fall inside the measuring aperture field of view to increase the signal-to-noise ratio and improve photometric precision.

8.3.3 A 10× to 40× objective may be necessary for effective transmission measurements of different specimen areas and conditions, and a objectives up to 100× can be used for measurements involving very small particles and pigment particle identification.

8.4 In transmission, the areas selected for analysis shall be as homogeneous as possible and uniform in size for the questioned and known samples. Avoid large particles such as effect pigments. It is also necessary that the sample thin section be as flat as possible to avoid stray (off-axis) light effects.

8.5 Sample areas selected for reflectance analysis shall be uniform in size and free of dust and scratches for the questioned and known samples.

8.6 The reflectance background reference scan shall be made using a white standard $BaSO_4$ or MgO, or a white unglazed ceramic tile, or commercial grade opal glass that are standardized. The background scan should be acquired before scanning samples. It is recommended that a new reference spectrum be measured for each new reflectance sample. This recommendation is a precaution to avoid spectral errors caused by short-term instrument instability.

8.7 For transmission measurements, it is necessary to run a new background reference scan for each new microscope slide and cover slip preparation and for each new aperture configuration.

8.8 The transmission or reflectance background and sample scans shall be made using all the same parameters (that is, objective(s), aperture area(s), lamp voltage, scan/spectrum averaging, step width, resolution, or sample focal plane).

9. Spectral Comparison and Interpretation

9.1 It is worth repeating the general caution that for comparative color examinations a basic rule must be complied with: the known sample(s) must originate from an area as near as possible to the suspected source point of the questioned sample(s). Differences may arise in top surface reflectance measurements of paint samples from the same vehicle or other painted surfaces because of differences in sunlight exposure, weathering conditions, or repaired areas. Transmission measurements can also exhibit variations for the same reasons.

9.2 Spectra can be compared by overlaying them on a light box or plotting them sequentially on the same graph. Mean value spectra that are generated from several scans of each sample and are bracketed by curves showing the ± 1 or more standard deviations of the mean sets are also suitable for comparison. Three standard deviation units will cover ~99 % of the expected sample variation under normal distribution conditions.

9.3 Spectra can be displayed and compared in percent reflectance or percent transmittance formats because some special features can only be seen in one or the other form. When comparison of percent transmittance spectra does not discriminate between those spectra, they shall be compared in absorbance format, which may provide additional information. In accordance with Beer's law, only absorbance spectra yield a linear variation with the concentration of the analyte. If questioned and reference samples contain more than one component and, therefore, more than one absorption band, the absorbance spectra then provide relative quantitative data about the concentration of the components. Spectra with single absorption bands that are displayed in absorbance can provide relative quantitative data about the concentration of a component in different samples of the same thickness.

9.4 Ideally, known and questioned spectra shall be identical if the samples are to be considered indistinguishable. In practice, known or questioned sample spectra may exhibit shape variations over different regions of the sample. If no questioned sample spectra fall within the range of variation exhibited by the known sample spectra, the known sample shall be eliminated as a potential source.

9.5 *Fundamental Steps of Comparison and Interpretation:*

9.5.1 The analyst shall gain appreciation for, and document, the range and variety of curve shapes expressed in known samples by studying them thoroughly in both percent transmittance and absorbance formats. Standard deviation curves can prove particularly useful in these instances for estimating the known sample variation range. First and second derivative functions of the spectra can also assist in identifying inflection points and aid in the discrimination of metameric samples. Effective use of derivative functions requires that spectra have low noise levels or high signal-to-noise ratios.

9.5.2 The known and questioned sample comparison begins with the examination of the whole spectrum, followed by critical examination of each specific peak. The comparison shall include examination of peak shape, minima, maxima, inflection points, troughs, shoulders, and the curves or slopes between peaks.

9.5.3 Known and questioned absorbance spectra shall overlay uniformly within the range exhibited by the known sample, if the known is to be considered a possible source of the questioned sample. The analyst can expect the absorbance spectra to have identical shapes, although they might not exactly superimpose. Variations in spectra will be minimized if the samples are embedded side by side and measured in transmission on the same thin section.

9.5.4 If paint samples are measured in reflectance, and both samples are not oriented normal to the optical axis of the microscope, variations may be observed in their spectra. The reflectance differences are generally seen as variations in reflectance intensity rather than in curve slope(s) or absorbance cutoff point(s). Multiple sampling/scan setups may resolve these variations. With some samples, such as non-effect pigmented paints, variations in spectra will be minimized if the samples are embedded side by side and smoothed by cutting or polishing.

10. Colorimetry Values Comparison and Interpretation

10.1 Colorimetry can be used for preliminary comparisons between samples, but its main application is in the automatic searching of databases. An automotive paint database containing colorimetry values can yield information concerning the possible make(s), model(s), and manufacturing year(s) of a vehicle involved in hit-and-run cases.

10.2 Comparisons can be carried out on mean chromaticity values. It is also important to compare the ranges of chromaticity values between the questioned and known samples. When the range of chromaticity values is greater in one sample than the other, it may be an indication that the questioned sample did not originate from the same source as the known sample, even in situations in which the mean values are the same.

10.3 The analyst shall not rely solely on colorimetry results when making comparisons. A spectral comparison shall always be undertaken because different curves, such as those from metameric samples, can yield similar chromaticity coordinates.

10.4 Error ellipses shall not be used as an evaluation tool for comparisons because they can vary dramatically with sample size, heterogeneity, and the presence of effect pigments.

11. Conclusions

11.1 The results of paint comparisons performed by MSP are typically expressed as one of three general concepts: indistinguishable, distinguishable, or inconclusive. It is important to understand that these results refer only to the comparison of MSP spectra and not to a complete paint examination.

11.1.1 *Indistinguishable*—When comparative analyses do not demonstrate significant differences between the samples, a statement can be made that no differences were indicated within the discriminatory limits of the analytical method.

11.1.2 *Distinguishable*—When comparative analyses demonstrate one or more significant differences between samples, a statement of dissimilarity can be made.

11.1.3 *Inconclusive*—The comparative analyses of some samples, such as damaged or contaminated samples or both, may yield results in which the differences may or may not be significant. Therefore, no definitive conclusion can be drawn.

12. Databases

12.1 Some agencies have created their own databases that are specific for automotive paint top coats/color coats. Their intended use is to assist in identifying a certain color and, thus a possible make of vehicle, by comparing measured chromaticity coordinates with the data from known samples. It is common for these databases to be compiled using a commercial/industrial reflectance spectrophotometer with large measuring fields of up to 5 cm^2. Samples that appear homogeneous using the large measuring areas of these instruments may be heterogeneous at the microscopical measurement level. Variations in topcoat color, and the fact that questioned samples are generally measured with a different instrument than that used to produce the database, can lead to erroneous associations and missed associations. Therefore, to compare samples measured with MSPs to a database compiled using these commercial/industrial instruments, mathematical corrections must be used.

12.2 Database comparisons shall only be used as a complementary technique to other analytical methods.

13. Report

13.1 Subject to a laboratory's procedures, case notes shall include:

13.1.1 All of the instrumental data used to reach a conclusion,

13.1.2 A unique sample designation,

13.1.3 A unique laboratory number,

13.1.4 A description of the evidence analyzed by MSP,

13.1.5 A description of the sample preparation,

13.1.6 Analytical instrumentation used, and

13.1.7 Operating parameters.

13.2 All data shall include the operator's name/initials and the date of analysis.

14. Keywords

14.1 color; color measurement; forensic paint analysis; microspectrophotometry; MSP